Meeting Report of Immunology and Skin Disease: New Perspectives

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In March 2007, a course entitled "Immunology and Skin Disease: New Perspectives" was given in Boston, Massachusetts.* In attendance were 180 physicians and scientists from 22 countries. The course, organized by Thomas Kupper, Robert Fuhlbrigge, and Rachael Clark of the Harvard Skin Disease Research Center in Boston, covered new developments in the field of cutaneous immunology. The key findings reported at the meeting are summarized here.

The course began with a breakfast session during which Ulrich von Andrian (Harvard University, Boston, Massachusetts) presented his studies on the imaging of adaptive immune responses in skin-draining lymph nodes. Using multiphoton microscopy of popliteal lymph nodes in live, anesthetized mice, Dr. von Andrian imaged the interactions of T and B cells with antigen-presenting cells (APCs) and found that priming of naive T cells occurs in three distinct phases. Using a mouse tumor model, he demonstrated that regulatory T (T-reg) cells did not block proliferation of tumor-specific CD8 cells but did prevent them from degranulating, blocking their ability to kill. *In vivo* imaging studies such as these provide the first glimpses into T-cell behavior within the lymph nodes and other tissues.

Thomas Kupper (Harvard University, Boston) outlined the objectives of the course and provided a brief introduction to skin immunity, highlighting recent findings by members of the Harvard Skin Disease Research Center.

Polly Matzinger (National Institute of Allergy and Infectious Diseases (NIAID), Bethesda, Maryland) presented updates to her danger model of the immune response. She discussed a common molecular feature of those stimuli that are interpreted as danger signals by the immune system: hydrophobic portions of molecules (Hyppos). Hyppos are normally sequestered within membranes or proteins, and their abrupt exposure as a result of injury is interpreted as a danger signal. Although the receptors on dendritic cells can react to microbial nucleic acids and proteins, Dr. Matzinger hypothesizes that these receptors evolved first to recognize endogenous products of cellular injury. This puts the injured tissue in control of the initiation of an immune response. Dr. Matzinger also believes that tissues determine the type of immune response to pathogens. As examples, she used "privileged sites," such as the eye, where transplants are not rejected. Although these sites have been thought to be off limits to the immune system, it is clear that immunity occurs in these tissues, including production of protective IgA antibody. The lack of a delayed-type hypersensitivity (DTH) response, as measured by the failure to reject an organ transplant, does not mean that there is no immunity at these sites but rather that it is switched away from potentially destructive DTH reactions toward less destructive classes of immunity. Dr. Matzinger proposes that DTH (Th1) responses, which are potentially very destructive to tissues, are not the default pathway of the immune system but are instead employed as a last resort to clear pathogens resistant to other responses. She suggested that each tissue has a default set of instructions that it communicates to the immune system (usually by instructing local dendritic cells) to ensure that a local immune response can clear a pathogen without destroying the local tissue in the process.

The cutaneous innate immune response encompasses many diverse elements, ranging from the simple barrier function of the skin to more complex systems, such as the complement cascade. Antimicrobial peptides (AMPs) are one of the skin's first lines of defense against pathogens. Richard Gallo (University of California, San Diego) summarized work in this field, noting that more than 20 AMPs have been identified in skin. These molecules have varied structures, are expressed by many skin cell types, and often have multiple biological functions. Dr. Gallo has found that differentially processed forms of the same cathelicidin either kill bacteria (the shorter form) or serve as pro-inflammatory danger signals (the longer form). In rosacea, he has found both an accumulation of the substrate for cathelicidin and an overexpression of the enzyme that converts it into the longer, pro-inflammatory molecule. The result is high production of a long form of cathelicidin that leads to inflammation. Last, Dr. Gallo discussed his surprising finding that cathelicidin production by keratinocytes is regulated by vitamin D. Wounding or other insults to the skin leads to local production of active vitamin D in keratinocytes, which induces cathelicidin production, along with upregulation of CD14 and TLR2.

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APCs are another critical element of the skin's innate immune system. Dendritic cells (DCs) are particularly important because they can stimulate naive T cells, thus initiating a primary immune response. In addition to stimulation, DCs pass on to T cells additional information about the nature of the pathologic insult and the tissue in which it was encountered. These signals affect the type of immune response that is induced and the expression of tissue-homing addressins that affect future T-cell migration patterns. Mark Udey (National Cancer Institute, Bethesda, Maryland) summarized recent findings in DC biology. Knockout mice deficient in Langerhans cells showed that these cells are not required for contact sensitivity responses. Models of cutaneous leishmaniasis demonstrated the important role of IL-12 production in the generation of a Th1 response. Severe HSV infections in DC-deficient mice highlighted the role of DCs in controlling this infection. Last, recent work in mice suggested that skin-derived APCs transport antigen to the draining lymph nodes, but, surprisingly, the antigen is then handed off to $CD8^+$ lymphoid DCs for presentation to T cells. Whether this occurs in humans remains to be seen.

Autoimmunity results when the immune system mistakenly interprets self antigens as dangerous. Ann Marshak-Rothstein (Boston University) addressed the role of toll-like receptors (TLRs) in autoimmune disease. She observed that targets of autoantigens serve as adjuvants by interacting with TLRs and triggering B-cell autoimmunity. CpG-rich stretches of mammalian (self) DNA are internalized into B cells and activate TLR9, leading to proliferation of these cells and the production of autoantibodies. In a similar way, mammalian RNA can activate TLR7 and lead to autoantibody production. Dr. Marshak-Rothstein suggested that women may be more susceptible to autoimmune diseases because the TLR7 gene is located on the X chromosome and the second copy of this gene may be incompletely inactivated by lyonization. Thus, increased TLR7 gene dosage may lead to an increased risk of autoimmunity. Her work highlights the important finding that cellular debris can activate endogenous TLRs, triggering and maintaining autoimmune disease.

NKT cells are thought to recognize glycolipid antigens presented by the CD1 family of molecules. The role of CD1d-restricted NKT cells in the immune response was summarized by Mark Exley (Harvard University, Boston). NKT cells rapidly produce large amounts of anti- and/or pro-inflammatory cytokines and chemokines. The activation of invariant NKT cells transiently protects against numerous diseases by stimulating DCs, APCs, and other lymphocytes, and these cells are needed for optimal responses to certain viruses and cancers. Th1-type invariant NKT can provide anti-tumor immunity, whereas Th2-type noninvariant NKT and T-reg cells can suppress anti-tumor responses. In cancer patients receiving IL-2 immunotherapy, invariant NKT cells behaved differently from other T-cell subsets and were more similar to myeloid DC1. Dr. Exley's work suggests that expansion of the invariant NKT cell pool or activation of these cells could lead to improved immunotherapies for cancer.

T and B cells express antigen-specific receptors and comprise what is termed the adaptive immune system. These cells have the ability to respond to virtually any antigen and can retain memory of past encounters. However, getting the right cells to the right place at the right time is a logistical challenge for the immune system. Robert Fuhlbrigge (Harvard University, Boston) discussed the critical role of cell homing to this process. He explained that tissue-specific "addressins" (adhesion molecules and chemotactic factors) support the specific and coordinated recruitment of cells into particular sites at different phases of the immune response. For example, T cells that first encounter their antigens in skin-draining lymph nodes develop expression of skin addressins, including CLA and CCR4, and home preferentially back to the skin in the future. Dr. Fuhlbrigge has discovered that properly glycosylated forms of PSGL-1 and CD43 both serve as ligands for E-selectin that support the entry of T cells into the skin under both normal and inflamed conditions. He presented evidence for an additional, as yet uncharacterized E-selectin ligand on human T cells. A clearer understanding of the mechanisms of immune surveillance and tissue-specific immune responses in skin is critical for the development of novel therapeutic agents and more effective vaccines.

Frances Lund (Trudeau Institute, Saranac Lake, New York) concluded the first day of the meeting by presenting groundbreaking work demonstrating new roles for B cells in inflammatory diseases. Antibody production was previously thought to be the main function of B cells. However, depletion of B cells in patients profoundly reduces the clinical symptoms of multiple autoimmune diseases, despite the continued presence of autoantibodies. Dr. Lund reported new findings that mouse B cells can produce an array of inflammatory cytokines in response to signals provided by T cells and that the types of cytokines produced are dependent on the environment in which the B cells were initially primed. B cells activated by Th1 cells or Th1-type pathogens (Be1 cells) made a different array of cytokines than B cells activated by Th2 cells or Th2-type pathogens (Be2 cells). In addition, Be1 and Be2 cells were potent APCs that efficiently promoted the development and expansion of effector T cells. T cells primed by these biased B cells were in turn induced to produce a similar profile of inflammatory cytokines. Thus, effector B cells can sustain and amplify preexisting inflammatory T-cell responses and have the potential to exacerbate T-cell-dependent autoimmunity. Cytokines produced by B cells also regulated the development and architecture of tertiary lymphoid tissues found in autoimmune lesions and induced the differentiation of B cells into long-lived plasma cells. Cytokine-producing B cells are thus important regulatory cells that can profoundly influence immune responses to pathogens and autoantigens. Last, Dr. Lund demonstrated in a mouse model of chronic nematode infection that B cells regulated the development and maintenance of antigen-specific $CD4^+$ Th2 cells and produced cytokines needed during the effector phase of the

immune response. Together, her findings demonstrate that B cells have multiple effector functions that are critically important during immune responses to pathogens and likely play a role in autoimmunity as well.

The second day of the conference began with a lively discussion by Arlene Sharpe (Harvard University, Boston) of the role that molecules in the B7:CD28 family play in T-cell activation and tolerance. The B7:CD28 family of receptors can either inhibit or enhance T-cell responses. Dr. Sharpe generated knockout mice deficient in the expression of PD-L1 and PD-L2, ligands for the recently characterized costimulatory molecule PD-1. She reported that islet cells express PD-1 and that nonobese diabetic mice develop accelerated diabetes in the absence of PD-1 ligands. Protection was restored when PD-L1 was expressed by the tissues but not by immune cells, suggesting that PD-L1 expression by tissues may shield them from autoimmune attack. Additional work suggests that microorganisms may also utilize the PD-1 pathway to escape host immunity, allowing for chronic infection.

Skin resident T cells—the T cells that reside within noninflamed human skin—play a critical role in providing immunosurveillance of the skin, contribute to psoriasis, and likely play a role in other inflammatory skin disorders as well. Rachael Clark (Harvard University, Boston) presented her work on the isolation and study of these cells. She has found that normal-appearing human skin is populated by 1 million memory T cells/cm^2, suggesting that there are nearly twice as many T cells in normal skin than are present in the entire circulation. These cells express the skin homing addressins CLA and CCR4, indicating that homing to both normal and inflamed skin probably occurs via similar mechanisms. Dr. Clark has discovered that human skin also contains a population of FOXP3$^+$ T-reg cells. These cells expand under conditions similar to those in inflamed skin, suggesting that local proliferation of T-reg cells within the skin may serve as a brake for cutaneous inflammation. Last, Dr. Clark described her recent work characterizing the mechanisms by which squamous cell carcinomas (SCCs) of the skin evade the immune system. She found evidence that SCCs evade the immune response by stealth and by suppression, subverting the normal processes of tissue-specific T-cell homing and tolerance induction to remain undetected by the immune system.

Regulatory T cells play a crucial role in the establishment and maintenance of self-tolerance. Ethan Shevac (NIAID, Bethesda, Maryland) reported that transfer of polyclonal FOXP3$^+$ T-reg cells into mice blocked the development of autoimmune gastritis. T-reg cells did not block proliferation of specific effector T cells but blocked their differentiation into pathogenic Th1-type effector cells. He found that TCR stimulation of mouse T cells in the presence of TGF-β induced FOXP3 expression, giving rise to induced T-reg cells that suppressed gastritis. These induced T-reg cells decreased the ability of DCs to present antigen, at least in part by downregulating expression of CD80 and CD86. Dr. Shevac has also observed FOXP3 expression in human T cells stimulated by TCR ligation and TGF-β but determined that these cells have no suppressive ability. He discussed the technical difficulties of working with T-reg cells, including the inadequacy of magnetic beads for isolating T-reg cells and differences in FOXP3 antibodies. His work suggests a role for FOXP3$^+$ T-reg cells in modifying the ability of autoreactive DCs to present antigen to pathogenic T cells.

Many inflammatory skin diseases are caused or exacerbated by T-cell activation and proliferation within the skin. The activation of T cells by APCs within the skin is central to understanding both normal immunity and the alterations of this process that give rise to inflammatory skin diseases. Stephan Grabbe (University of Essen, Germany) discussed his studies of the role that integrins play in DC–T-cell interactions. Within collagen gels, T cells and DCs form an immunologic synapse, inducing cytoskeletal remodeling within the DCs, which is required for full T-cell activation. Surprisingly, the β_2 integrins are not required for antigen presentation and DCs from normal mice express inactive β_2 integrins. Artificial activation of β_2 integrins with Mg^{2+} actually impairs DC activation of T cells, an effect mediated by Mac-1 (CD11b/CD18) on DC. Blockade of Mac-1 also increases macrophage activation of T cells. Dr. Grabbe observed that Mac-1 is known to bind to cellular debris and hypothesized that inactivation of Mac-1 in professional APCs may serve to protect against presentation of autoantigens and may thus protect against autoimmunity.

An understanding of DC physiology is also central to the development of more effective vaccines. Akira Takashima (University of Toledo, Ohio) reported the identification of novel DC stimulatory compounds by use of a high-throughput screening assay. Dr. Takashima has discovered that two common medications, colchicine and podophyllotoxin, have potent and previously unsuspected abilities to activate DCs. Findings from this work may lead to the development of a new class of DC-targeted vaccine adjuvants and immunostimulants.

The last segment of the conference focused on discussion of specific skin diseases, including graft-versus-host disease (GvHD), psoriasis, atopic dermatitis (AD), and cutaneous lymphomas. Some truly groundbreaking work was presented that emphasized the importance of dendritic cells in determining the host response.

James Krueger (Rockefeller University, New York) presented work that suggested a key role for TNF- and iNOS-producing dendritic cells in psoriasis. Dr. Krueger reported that these cells are key producers of IL-23 and IL-10, cytokines that stimulate Th17 T cells to produce IL-17 and IL-22. These cytokines in turn produce alterations in epidermal keratinocytes, leading to psoriasiform hyperplasia. One additional key observation made by Dr. Krueger was that psoriasis and AD share a great number of susceptibility genes, and the genes that differ between these disorders are those expressed by DCs. This suggests that a common set of genes predisposes individuals to the development of both disorders and whether a person develops psoriasis or AD may depend on the characteristics of his or her dendritic cells.

Thomas Bieber (University of Bonn, Germany) presented his groundbreaking and eminently useful research on AD. He reported that the earliest stages of AD in infants begin as a dermatitis lacking IgE-mediated specific immunity. This, together with an altered skin barrier, allows the immune system to become sensitized to environmental allergens, leading to skin inflammation and IgE-mediated allergy. Chronic itching and scratching then leads to a third phase of the disease, in which sensitivity to self proteins, or "autoallergens," sets up a patient for long-term AD that is actually a form of autoreactivity. Dr. Bieber also noted that calcineurin inhibitors may change the phenotype and function of epidermal DCs in AD, perhaps leading to resurgence in Langerhans cell-mediated tolerance within the skin. His work highlights the fact that a decrease in the skin's barrier function can set up patients for lifelong sensitization to both environmental and self antigens. His results suggest that good skin care and emollients, together with anti-inflammatory topical medications, not only improve symptoms but also may help stop the progression of allergic disease.

Paul Nghiem (University of Washington, Seattle) reviewed current knowledge about the diagnosis and management of GvHD. It can be very difficult to distinguish between drug eruptions and GvHD in transplant recipients, yet the treatments for these two problems are markedly different and untreated GvHD can be rapidly fatal. A recent decision analysis showed that, for populations of patients with a prior probability of GvHD of at least 30%, the best approach is to treat presumptively for GvHD and forgo a skin biopsy altogether. Skin biopsies simply cannot distinguish between the two conditions. Dr. Nghiem also reviewed recent findings suggesting that T-reg cells may be capable of attenuating or preventing GvHD after hematopoietic stem cell transplant. The number of T-reg cells in the blood falls with development of GvHD, and extracorporeal photopheresis, which increase the numbers of T-reg cells, is effective in treating the condition. Photopheresis may work by producing apoptotic debris that, when phagocytosed by DCs, induces them to adopt a tolerogenic phenotype. These tolerogenic DCs may then induce the formation of T-reg cells.

The final session was a thought-provoking discussion of cutaneous T-cell lymphoma (CTCL) by Madeleine Duvic (University of Texas, Houston). CTCL remains a somewhat enigmatic group of malignancies, and many hypotheses have been put forth to explain the underlying physiology. Malignant T cells in CTCL have defective apoptosis that may allow them to accumulate within skin lesions. It may be that chronic antigen stimulation drives the proliferation of T cells that eventually develop genetic mutations, giving rise to the malignant clones that characterize later stages of disease. Dr. Duvic has demonstrated colonization of CTCL patients with superantigen-producing *Staphylococcus aureus* and expansion of superantigen-reactive T cells in these patients. The role of T-reg cells in CTCL is controversial. It has been suggested that CTCL is a malignancy of T-reg cells, yet photopheresis, which increases the number of these cells, is effective in the treatment of the disease. A deeper understanding of CTCL awaits the development of a unifying hypothesis capable of explaining the many confusing aspects of this group of diseases.

The course was then concluded by Dr. Kupper with thanks to the organizers, speakers, and attendees. The course will be held again in two years, on a date to be announced.

**Immunology and Skin Disease: New Perspectives was held at the Fairmont Copley Plaza Hotel in Boston, Massachusetts, USA, 22–24 March 2007.*